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Effects of N-methyl-D,L-aspartate on LH, GH, and testosterone secretion in goat bucks maintained under long or short photoperiods¹

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ABSTRACT: Photoperiod modulates reproduction in goats. We tested the hypothesis that the excitatory glutamatergic tone is reduced in the photoinhibited goat. The objectives of this study were to determine the effect of photoperiod and glutamatergic stimulation on LH, GH, and testosterone (T) secretion in goat bucks. Eight mature, intact bucks were used in two simultaneous 4 × 4 Latin square designs. Variables were two photoperiod regimens (short day; SD, 10 h light:14 h dark, n = 4; vs long day; LD, 16 h light:8 h dark, n = 4) and four doses of N-methyl-D,L-aspartate (NMA; 0, 1, 2 and 4 mg/kg BW, i.v.). Venous blood was obtained for 2 h before and after NMA injection, followed by GnRH injection and then a final 1 h of sampling. Injection of NMA increased ($P < 0.002$) LH secretion within 20 min. This increase was sustained for 120 min, but the response was most pronounced in LD goats. The increase in mean LH was associated with a concomitant dose-

dependent increase in pulse frequency ($P < 0.006$). However, NMA treatment had no effect ($P > 0.10$) on LH pulse amplitude. The release of LH after injection of GnRH was not affected by photoperiod. Exposure of bucks to LD reduced T secretion relative to that of SD bucks ($P < 0.01$). However, GH secretion was enhanced in LD bucks ($P < 0.001$). The response of GH to NMA was dependent on photoperiod history. A highly significant immediate and sustained increase ($P < 0.001$) was observed in LD but not in SD bucks within 10 min. Overall, a dose-dependent increase ($P < 0.01$) in T secretion was stimulated by NMA in both LD and SD bucks. These results indicate that NMA receptors may be involved in the regulation of LH, GH, and testosterone secretion in the goat. Furthermore, length of day influences GH secretion in the goat and NMA receptor activation had divergent effects on the secretion of this hormone.

Key Words: LH, GH, Testosterone, Aspartic Acid, Photoperiod, Goats

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Introduction

Photoperiod is the most important cue for regulating annual stimulatory and inhibitory rhythm of reproductive activity in the goat (Chemineau et al., 1992). Also, photoperiod is involved in the regulation of growth in some mammals. In goats, growth peaks occur in mid-to late summer (Walken-Brown et al., 1994, 1997), and supplemental lighting stimulates growth in cattle (Mosberg and Jonsson, 1996). The mechanism by which

photoperiod affects growth is not clear. The secretion of the major reproductive hormones, LH and FSH, together with GH, a component of the somatotrophic axis, is regulated by an array of neurotransmitters and neuromodulators. The excitatory amino acids (EAA), glutamate and aspartate, are major stimulatory neurotransmitters in the mammalian nervous system. Acting through both N-methyl-D-aspartate (NMDA) and non-NMDA receptors, these amino acids modulate the secretion of LH, GH, and prolactin (Estienne et al., 1990a,b; Lincoln and Wu, 1991; Ping et al., 1994; Barb et al., 1996; Jiang et al., 1997). Various studies in laboratory animals have suggested a role for NMDA receptors in the control of seasonal breeding and the stimulatory effect of NMA on LH secretion is amplified in seasonally anestrus rams (Lincoln and Wu, 1991). The possibility exists that changes in the secretory pattern of EAA or NMDA receptor function may be involved in the regulation of seasonal reproduction. Specifically, the role played by these agents in the control of reproduction and growth in the goat has not been documented.

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The objective of our study was to test the hypothesis that a reduction in stimulatory glutamatergic input to the reproductive axis is a major component of the inhibitory effects of long photoperiods. Further, few studies have investigated the regulation of LH secretion in the goat. Accordingly, we investigated the effects of N-methyl-D-L-aspartate (NMA) on LH, GH, and testosterone secretion in goats kept under either long or short photoperiods.

Materials and Methods

Animals and Treatments. Eight mature, intact dairy goat bucks were maintained either in a photoperiod room and subjected to a regimen of 16 h of light and 8 h of darkness (long day [LD]; $n = 4$) or under ambient photoperiod conditions (short day; SD), average photoperiod, 10 h of light and 14 h of darkness; $n = 4$) beginning in December. The experiment was conducted in January at Fort Valley, GA (32°, 33' N) after a minimum of 30 d of exposure to LD and SD photoperiods. Bucks were used in two simultaneous 4×4 Latin square designs involving both photoperiods (LD vs SD). Each square consisted of four animals and four doses of NMA (0, 1, 2, and 4 mg/kg BW in saline i.v.).

Blood was obtained by jugular venipuncture at 10-min intervals for 2 h, after which the vehicle or NMA (Sigma Chemical, St. Louis, MO) was injected i.v. and sampling continued for another 2 h, first at 5-min intervals for the first 20 min and then at 10-min intervals for the next 100 min. This was followed by a 10 μ g GnRH i.v. injection and blood samples were collected at 15-min intervals for an additional h. The sampling events were separated by at least 3 d. Plasma was harvested and stored at -20°C until analysis for LH, GH, and testosterone by RIA.

Radioimmunoassays. Plasma LH and GH concentrations were determined by RIA using kits supplied by National Institute of Digestive Diabetes and Kidney Disease (NIDDK). Luteinizing hormone concentrations were determined as described previously (Gazal et al., 1998) with the modification that the antigen-antibody complex was separated using 6% polyethylene glycol solution in 0.01 M PBS. Intra- and interassay coefficients of variation were 3 and 11%, respectively. Plasma GH concentrations were determined in samples collected for the first 4 h using procedures described previously (Ryan et al., 1994) with the following modifications. Briefly, 300 μ L of plasma, 200 μ L of PBS – 1% BSA, and 200 μ L of rabbit anti-ovine GH (AF-PCO123080) in PBS-EDTA containing 1:400 NRS were added together in 12- \times -75-mm borosilicate tubes and incubated overnight at 4°C . Then, 100 μ L of [^{125}I]ovine GH (AFP12855B) containing 25,000 cpm was added and incubated for 24 h, followed by 200 μ L of sheep anti-rabbit gamma globulin and incubation for another 24 h. Finally, 200 μ L of 15% polyethylene glycol solution and 1 mL of 0.01 M PBS solutions were added to precipitate the antigen-antibody complexes. Plasma testosterone

one concentrations were determined only in samples obtained immediately before and at 30, 60, 120, and 180 min after NMA injection (the last sample at 60 min after GnRH challenge) using a commercial kit (Diagnostic Products, Los Angeles, CA). The sensitivities of the assays were 0.3 ng/mL of GH and 0.2 ng/mL of testosterone, respectively. Intra- and interassay coefficients of variation were 3 and 12% for GH and 2 and 3% for testosterone, respectively. Luteinizing hormone pulses were identified with Pulsefit 1.2 (Kushler and Brown, 1991).

Statistical Analyses. Data were subjected to the GLM split plot-in-time ANOVA procedure of SAS (SAS Inst. Inc., Cary, NC). The sampling was divided into five periods, I through V. Period I was a 120-min pretreatment, period II was the 30 min immediately after saline or NMA injection, period III was the next 30 min, period IV was the 2nd h after saline or NMA injection, and period V was the hour after GnRH injection (for analyses of LH and testosterone only). The statistical analysis was done for each period and the model included the main effects of photoperiod, dose, and photoperiod \times dose interactions. Where significant differences existed, least square contrasts were used for separation of means.

Results

Pretreatment plasma LH was below the level of detection in both SD and LD goats. In period II, NMA injection induced a dose-dependent increase ($P < 0.002$) in LH secretion. This increase was sustained for 120 min (Figures 1A and B). However, only the LD goats were responsive to the lowest dose of NMA, leading to a significant photoperiod \times dose interaction ($P = 0.002$). The same trend was observed in periods III and IV. Significant photoperiod effects were also observed in period III. The increase in mean LH secretion was associated with a concomitant dose-dependent increase in LH pulse frequency ($P = 0.006$) but NMA treatment had no effect on pulse amplitude. The NMA tended to increase ($P = 0.08$) LH pulse frequency and not pulse amplitude in LD bucks compared to SD animals (2.3 ± 0.3 vs 1.4 ± 0.3 pulses/4 h, respectively; data not shown). Treatment with GnRH markedly increased LH secretion in both SD and LD goats but the response was not affected by photoperiod ($P = 0.97$).

During the pretreatment period (period I), mean plasma GH concentrations were greater ($P < 0.001$) in LD than in SD bucks (Figure 2). Also, the pattern of GH response to NMA was dependent on photoperiod. An immediate, sustained increase ($P < 0.001$) was observed in LD bucks within 10 min (Figure 3A). The magnitude and duration of this increase was dependent on the dose of NMA, reaching significance ($P < 0.0001$) at the intermediate and high NMA doses. In contrast, NMA injection in SD bucks had no effect on plasma GH in period II. However, by period IV, both the intermediate and high NMA doses significantly increased GH

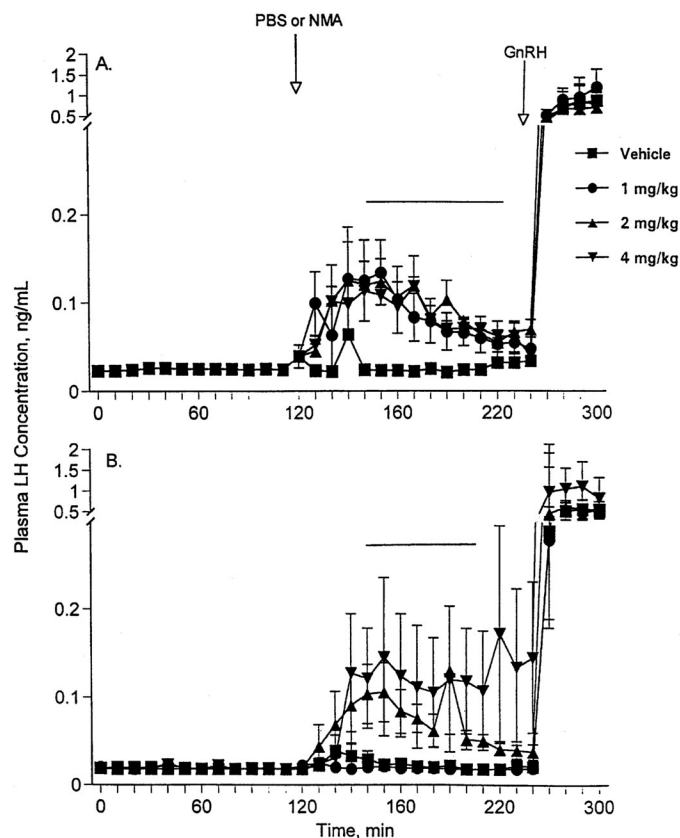


Figure 1. Plasma LH secretion in goat bucks exposed to (A) long photoperiod (top panel; 16 h light:8 h darkness, $n = 4$) and (B) short photoperiod (bottom panel; 10 h light:14 h darkness, $n = 4$). Blood samples were obtained at 10-min intervals for 2 h before and after N-methyl-D-L-aspartate injection at 0, 1, 2 and 4 mg/kg BW. GnRH (10 μ g) was injected i.v. and samples obtained for 1 h at 15-min intervals.

levels above those seen in saline-treated animals (Figure 3B).

Exposure of bucks to LD reduced ($P < 0.01$) plasma testosterone secretion relative to SD (Figure 2). Injection of NMA increased ($P < 0.01$) testosterone secretion in a dose-dependent fashion. This increase was independent of photoperiod (Figures 4A and B). Injection of GnRH stimulated testosterone secretion in saline-treated goats and in goats receiving the 1-mg dose of NMA. However, no further increases in testosterone were observed in goats that received higher doses of NMA (Figures 4A and B).

Discussion

The stimulatory effect of NMA on plasma LH in the present study, especially in the LD goats, agrees with previous findings in other mammals (Estienne et al., 1990b; Lincoln and Wu, 1991; Jansen et al., 1991). Therefore, tonic secretion of LH in the goat may be regulated in part by EAA. In a previous study (Lincoln and Wu, 1991), NMA stimulation of LH secretion was

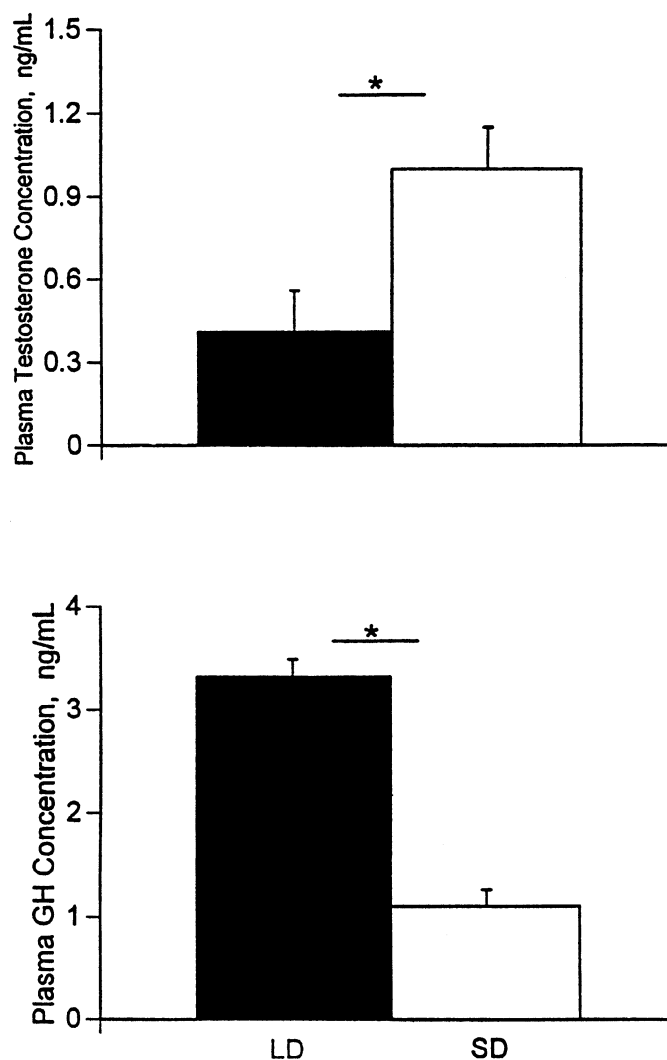


Figure 2. Main effects of photoperiod on plasma testosterone (top panel) and growth hormone (bottom panel) concentrations in goat bucks. Animals were exposed to 30 d of either short photoperiod (SD; 10 h light:14 h darkness) or long photoperiod (LD; 16 h light:8 h darkness), after which blood samples were obtained at 10-min intervals for 120 min. Bars with asterisks are significantly different, $P < 0.01$.

greater during seasonal anestrus than during the breeding season in the ram. Our results indicated a heightened NMA effect on LH secretion in LD goats. This occurred in spite of the similar preinjection LH levels in both groups of goats, suggesting both a diminished excitatory amino acid tone in the photoinhibitory LD state and a refractoriness to EAA stimulation under SD conditions. The reduced effectiveness of NMA in the SD goats may be related to the timing of the current study. By December, the breeding season is nearly over and therefore our bucks may have entered early stages of the nonbreeding season. In agreement with our results, a study in sheep suggested that NMA may act independently of photoperiod to influence LH secretion

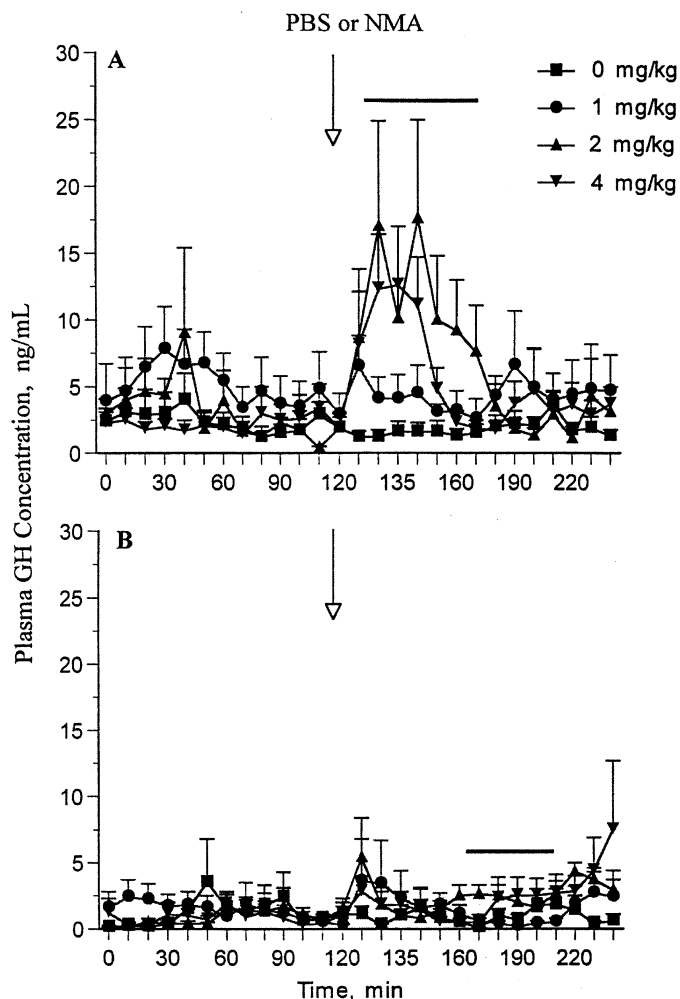


Figure 3. Effect of N-methyl-D-L-aspartate (NMA) on GH secretion in goats maintained under (A) long (top panel; 16 h light:8 h darkness; $n = 4$) or (B) short (bottom panel; 10 h light:14 h darkness; $n = 4$) photoperiods. Blood samples were obtained at 10-min intervals for 2 h before and after NMA injection at 0, 1, 2 and 4 mg/kg BW. Values are means \pm SE. Bar represents points of significant differences, $P < 0.05$.

(Viguie et al., 1995). The concomitant increase in LH pulse frequency suggests that NMA possibly acts at the hypothalamic level. N-methyl-D-L-aspartate stimulates GnRH release both in vitro (Gay and Plant, 1982) and in vivo (Viguie et al., 1995). These amino acids may also stimulate other neuromodulatory pathways, for example, neuropeptides (Bonavera et al., 1993) or catecholamines (Saitoh et al., 1991). Our current results indicate the need for further studies involving longer exposure to inhibitory photoperiods to enable a clear definition of a role for EAA in the regulation of LH secretion during the nonbreeding season in the goat.

The present results demonstrate that a long photoperiod enhances GH secretion and inhibits testosterone secretion in goats. This increase in GH may explain in part the increased growth in goats and other ruminants

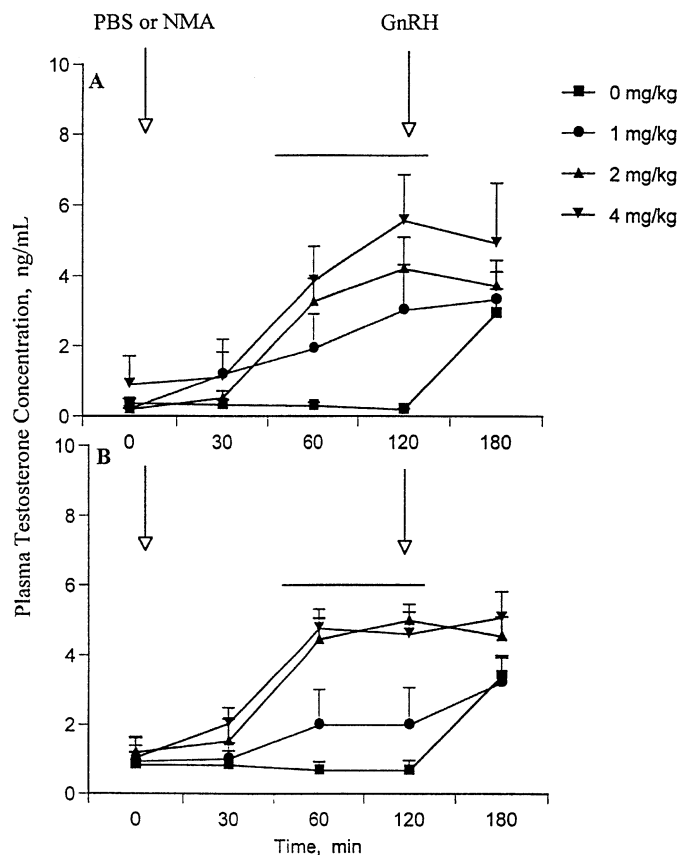


Figure 4. Effect of graded doses of N-methyl-D-L-aspartate (NMA) on mean plasma testosterone secretion in goats maintained under (A) long (top panel; 16 h light:8 h darkness; $n = 4$) or short (bottom panel; 10 h light:14 h darkness; $n = 4$) photoperiods. Testosterone was measured in blood samples obtained immediately before the injection of NMA at 0, 1, 2, and 4 mg/kg BW, and then at 30-min intervals for the next 120 min, and 60 min after injection of GnRH (10 μ g, i.v.). Bar represents points of significant differences, $P < 0.01$.

observed during the summer or under supplemental lighting (Walken-Brown et al., 1997). Evidence exists that GH is lactogenic in goats (Knight et al., 1990) and other ruminants (Sandles et al., 1988; Kann, 1997). Furthermore, milk production is increased during the summer months relative to other times of the year (Montaldo et al., 1997). Therefore, this increase may be mediated by the increased GH secretion induced by long photoperiod. The decrease in testosterone induced by long photoperiod in the current study is similar to earlier reports in the ram (Langford et al., 1987; Lincoln et al., 1996).

Testosterone secretion is regulated mainly by LH secretion from the anterior pituitary. In the present study, long photoperiod was inhibitory to LH secretion and resulted in decreased testosterone secretion. Injection of NMA markedly increased testosterone secretion within 30 min in this study, in agreement with results obtained in monkeys (Shahab et al., 1997). Injection

of NMA stimulated LH secretion in other ruminants (Estienne et al., 1990b; Lincoln and Wu, 1991; Jansen et al., 1991). Furthermore, a testosterone pulse occurred approximately 15 to 30 min after an LH pulse in goats (Coleman et al., 1998). Thus, the effect of NMA on testosterone secretion may be indirect, meaning that it is mediated by the NMA-induced increase LH secretion. The effect of NMA on testosterone was not affected by photoperiod. In sheep, NMA acted independently of photoperiod to influence LH secretion (Viguie et al., 1995), although other studies reported that NMA stimulation of LH secretion was greater during seasonal anestrus than during the breeding season (Lincoln and Wu, 1991). This lack of difference based on photoperiod may relate to the length of exposure, which was only 30 d in this study.

In agreement with previous findings in sheep (Estienne et al., 1989, 1990a) and pigs (Barb et al., 1992, 1996; Estienne et al., 1996), NMA administration markedly increased GH secretion for 30 to 60 min after NMA injection. This effect was markedly evident in LD goats. Because GH secretion is under both stimulatory and inhibitory regulation, it is likely that NMA acted by stimulating GHRH secretion. This hypothesis is supported by results obtained in the barrow and gilt, in which immunoneutralization of GHRH prevented the stimulation of GH secretion by NMA (Barb et al., 1996; Estienne et al., 1996). Furthermore, NMA treatment failed to stimulate GH secretion in rats with neonatally damaged arcuate nucleus, the site of GHRH-synthesizing cells. Unlike in normal rats (Zelena et al., 1998) and in vitro, somatostatin suppressed, whereas GHRH potentiated, the stimulatory effect of NMA on GH secretion (Niimi et al., 1994). Moreover, NMA may also act through endogenous opioid receptors as previously reported in the pig, in which naloxone pretreatment blunted the GH response to NMA injection (Chang et al., 1993). In sheep, however, there is no support for the involvement of EOP in NMA stimulation of GH secretion (Estienne et al., 1990a). Collectively, these results suggest a hypothalamic site of action.

Unlike LH and testosterone secretion, photoperiod influenced the response of GH to NMA injection. It is likely that other EAA receptors are involved. For example, non-NMDA receptors are involved in the regulation of GH secretion under conditions of short photoperiods. In support of this idea, photoperiod regulates activation of GABA receptors (Scott and Clarke, 1993). On-going studies are directed at determining the mechanism(s) by which photoperiod and NMA modulate adenohypophyseal hormone secretion in the goat.

Implications

Activation of N-methyl-D-L-aspartate (NMA) receptors produced divergent effects on the secretion of LH and GH secretion in goats, depending on the prevailing photoperiod. In goats exposed to long photoperiods, NMA increased GH secretion relative to those main-

tained under short photoperiods. However, response of LH to NMA was not as robust under the same photoperiod. However, the sustained increases in testosterone secretion indicate that excitatory amino acids may be involved in the physiological signaling of the pituitary-gonadal axis in the goat.

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